# THE PROBLEM OF NEURONAL REGENERATION IN THE CENTRAL NERVOUS SYSTEM

## II. THE INSERTION OF PERIPHERAL NERVE STUMPS INTO THE BRAIN

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In a previous communication (Clark, 1942) the results of implanting in the brain of portions of predegenerated sciatic nerve were recorded. In some experiments regenerating axons were found to have penetrated into the implant. They were very few, however, and it was concluded that, if the intrinsic fibres of the rabbit's brain have any regenerative capacity at all, it is relatively feeble in comparison with that of peripheral nerve fibres. On the other hand, the experiments provided a strong presumption that the regenerating fibres were derived, not from the new growth of intrinsic fibres of the brain, but from other sources such as vascular nerve fibres and peripheral fibres in the meninges or scalp tissues. In other words, no conclusive evidence was obtained for the view of neurohistologists such as Cajal (1928), Tello (1911), and Ortin & Arcaute (1913), that the tissues of a predegenerated peripheral nerve induce the active regeneration of nerve fibres in the central nervous system. In criticism of the experiments it may be justly argued that, since the portions of predegenerated nerve were taken from other animals (homografts), and since the vitality of their cellular elements would be seriously affected by the absence of all blood supply until they had become secondarily vascularized, the influence which they may otherwise exert on nerve regeneration would be at least greatly diminished. To overcome such difficulties a further series of experiments has been completed. In this series either the proximal or the distal stump of a cut peripheral nerve was directly inserted into the brain of the same animal. Thus the advantages of an autograft were combined with the maintenance of a vascular supply (even though the latter was probably diminished to some degree by the preliminary mobilization of the nerve). Further, the insertion into the brain of the proximal stump of a peripheral nerve permitted observations on the behaviour of axons growing from the cut end of the nerve when they come into relation with the tissues of the central nervous system.

### METHODS

The experiments were carried out on rabbits under intravenous nembutal anaesthesia, using strict aseptic precautions. In the insertion of the proximal stump of a peripheral nerve, the facial nerve was employed in five animals. An incision was first made along the course of the nerve over the surface of the masseter muscle. The nerve was lifted up and cut as far forwards as possible. Another incision was then made over the temporal region. The temporal muscle was separated from its bony origin with a raspatory, and a small trephine hole was drilled in the underlying skull. The proximal stump of the facial nerve was then drawn under the skin up to the position of the trephine hole, and with a pair of fine forceps it was pushed through the dura mater and into the substance of the brain. The inserted nerve was sealed in position by a few drops of concentrated plasma (for the preparation of which I am grateful to my colleague Dr Medawar). The incisions were then closed. In some cases it was found that the parotid duct, which is closely adherent to the facial nerve, had also been inserted into the brain. As will be seen, the proliferation of its lining epithelium in the brain tissues was not without interest. In three animals the facial nerve was crushed at the most proximal part of its facial course before insertion. For the insertion of the distal stump of a peripheral nerve, the occipital nerve was used in four animals. This nerve, which is small but of convenient size, is the posterior primary ramus of the second cervical nerve. It extends up the back of the neck to the occipital region of the head and finally passes on to the medial margin of the ear. The nerve was exposed by a longitudinal incision to one side of the midline. It was cut at the point where it emerges from the nuchal musculature, and the proximal end of the distal stump was inserted into the occipital region of the cerebral hemisphere through a small trephine hole in the skull. Since the nerve was long enough to be inserted into position without tension, sealing with plasma was not necessary. The incision was closed in the usual way. In one animal, the inserted nerve was exposed at a second operation one week before death and sectioned close to the skull to ensure degeneration of any regenerated axons which might have grown down in a recurrent direction.

The animals were kept alive for periods varying from 1 to 6 weeks, and were killed by decapitation. In each experiment a block containing the site of insertion was removed from the brain and serial sections were cut at a thickness of $10\mu$. In most cases the block was treated by a pyridine-silver technique; material of two of the experiments was stained by Bodian's protargol method.

## EXPERIMENTAL RESULTS

*R* 230. Proximal stump of facial nerve inserted into brain, and animal killed 3 weeks later. Pyridine-silver sections show that the nerve extends through the cortex of the parietal lobe, traverses the medial margin of the lateral ventricle, and enters the underlying subicular cortex. The main cut end of the stump is imbedded in the white matter adjacent to the hippocampus. Although the nerve had not been crushed at the operation, the axons in the implanted portion had evidently undergone complete degeneration and are replaced by regenerated axons, which are present in great abundance. In most of the fasciculi the growth of the regenerated axons has been obstructed by fibrous tissue at their cut ends, and here the fibres form a tangled skein from which they extend their growth by running spirally round the inner aspect of the perineural sheath (Pl. 1, fig. 5). At the free end of the main stump of the implanted nerve there has been a considerable local reaction in the adjacent tissues of the brain; a circumscribed zone of granulation tissue, containing many macrophages and an infiltration of closely packed small round cells, extends down for a short distance into the subicular cortex. Penetrating into this zone of inflammatory tissue are numerous strands of Schwann cells which have grown out from the cut end of one of the fasciculi of the facial nerve; they form in some cases relatively thick bundles of closely packed cells and in others fine chains of single Schwann cells, and they are disposed in a loose plexiform arrangement. Extending out along many of these strands are fasciculi of actively growing axons which can be seen streaming out from the cut end of the implanted nerve (Pl. 1, fig. 1). They run for a distance of about 1 mm. into the granulation tissue and finally terminate in fine beaded fibres which cling closely to the fine terminal strands of Schwann cells (Pl. 1, fig. 2). In some cases the free ends of these regenerated fibres appear to run into the granulation tissue without accompanying Schwann cells, but the histological picture as a whole suggests very forcibly that the proliferating strands of Schwann cells (many of which have no axons in relation to them) serve as guides for the subsequent outgrowths of regenerating nerve fibres.

The cellular infiltration of the subcortical white matter in the neighbourhood of the stump of the implanted nerve has led to the local destruction and disappearance in this situation of the fibre plexuses of the brain. However, scattered fibres are found still persisting in the marginal parts of the infiltrated zone, particularly along the course of blood vessels where, presumably, their survival is facilitated. In some sections the extensions of such fibres from the adjacent intact white matter along blood vessels into the zone of cellular infiltration gives the appearance of an actual outgrowth of new fibres from the white matter of the brain towards the Schwann tissue at the cut end of the implanted nerve. These fibres, however, examined with a high-power objective, show none of the characteristic appearances of actively regenerating fibres. On the contrary, at the margins of the infiltrated zone, the persisting fibres of the white matter show many signs of degeneration, such as fragmentation, uneven thickenings and the formation of conspicuous globular retraction bulbs. Compared with the regenerating fibres of the implanted nerves, also, they are but lightly impregnated, and they show no tendency to extend out along adjacent strands of Schwann cells. At one point in their farthest extension the outgrowing facial nerve fibres actually intermingle in the granulation tissue with persistent fibres of the white matter (Pl. 1, fig. 3), and it may be difficult by superficial inspection of one section alone to determine the source and identity of some of them. However, a detailed study of the serial sections indicates that all those fibres which show characteristic signs of active regeneration and which extend along strands of Schwann cells are derived from the fibres of the facial nerve.

The proximal part of the outgrowth of axons and Schwann cells from the stump of the implanted nerve is in direct contact on one side with the white matter of the alveus, the lateral margin of which it penetrates. Here there is no inflammatory reaction in the tissue of the brain, and the alvear fibres are immediately contiguous with the proliferating Schwann elements at the end of the facial nerve with no intervening tissue (Pl. 1, fig. 4). Moreover, some strands of Schwann cells actually penetrate in among the fibres of the white matter. However, the latter show no tendency to extend along the Schwann cells with which they are in direct contact. The plexus of alvear fibres as a whole presents an abrupt margin where it meets the adjacent proliferating Schwann tissue, and even where the fibres almost touch outgrowing fibres from the implanted nerve they still show no tendency to make use of the strands of Schwann cells available to them for regenerative extension.

As the main part of the implanted nerve passes through the superficial layer of parietal cortex, the cut ends of some of the smaller fasciculi are seen to be imbedded in the cortex and also in the subcortical white matter. From these stumps regenerating fibres have grown out, but they do not penetrate into the normal tissue of the brain. They wander down closely hugging the surface of the perineural sheaths of adjacent fasciculi and are separated from the fibre plexuses of the cortex and white matter by a thin secondary capsule of fibrous elements derived from the brain tissues. Some of the outgrowing fibres have found their way into the substance of this capsule. On the other hand, the fibres of the adjacent cortex and white matter of the brain show no tendency to extend out towards the implanted nerve, nor are they found in the fibrous layer separating them from the latter.

*R* 233. Proximal stump of facial nerve inserted into brain after crushing, and animal killed 4 weeks later. Pyridine-silver sections show that the nerve extends through the parietal region of the cortex and the subcortical white matter, and through the dorsal part of the underlying hippocampus. Its free end lies on the inner surface of the brain imbedded in the leptomeninges between the hippocampus laterally and the optic tract medially (Pl. 2, fig. 7). Vigorous regeneration of the facial nerve fibres has occurred, the individual fasciculi being filled with large numbers of actively growing axons which are deeply impregnated. The tissues of the brain show remarkably little reaction.

At the free cut end of the nerve, many regenerating axons have emerged from the stump. Some of these become lost in the pial tissue covering the stump, a few running for a short distance alongside small pial veins. Most of them curl back up the surface of the nerve stump, pushing their way between the latter and the adjacent surfaces of the brain.

On the medial side they extend up on the surface of the hippocampus and on the lateral side on the surface of the optic tract (Pl. 1, fig. 6; Pl. 2, fig. 9). The superficial fibres of the latter, it should be noted, have been slightly traumatized by the lesion. These outgrowing fibres are accompanied by elongated cellular elements which appear to be proliferated Schwann cells, but it is not possible to identify them with certainty. Although the regenerating axons run alongside the alvear fibres of the hippocampus and the fibres of the optic tract, in neither case do these fibres of the brain show any tendency to deviate from this normal position and to extend out towards the regenerating fasciculi. In their passage through the subcortical white matter, some superficial fasciculi of the implanted nerve are in almost immediate contact with the former, and at one point the regenerating facial nerve fibres are separated from the fibre plexuses of the white matter by a distance of not more than 15$\mu$ (Pl. 2, fig. 8). Yet the fibres of the white matter show no evidence of new growth.

*R* 229. Proximal stump of facial nerve inserted into brain after crushing, and animal killed 3 weeks later. Pyridine-silver sections show that the nerve penetrates through the parietal area of the cortex and the subcortical white matter to reach the ventricular cavity. The cut end of the nerve lies free within the lateral ventricle in close relation to the ventricular surface of caudate nucleus, the fimbria and choroid plexus. The parotid duct was found to have been inadvertently implanted with the facial nerve. In the cortex and subcortical white matter immediately adjacent to the nerve there is considerable inflammatory reaction, accompanied by local necrosis and infiltration with macrophages. Regeneration of the facial nerve fibres has occurred, though not so abundantly as in the two previous experiments. Many of the regenerated fibres have reached the cut end of the implanted nerve, and most of them here turn aside and wander round within the confines of the perineural sheaths. At one point, however, some have grown out into the layer of granulation tissue which covers the nerve stump where it lies within the lateral ventricle. Of these, a few extend their course to the surface of the fimbria and even penetrate in among the most superficial fibres of this tract. Of particular note, however, are two or three stout fibres which, leaving the cut end of the implanted nerve, traverse a layer of granulation tissue to reach the choroid plexus. In relation to this structure they extend downwards and break up into a leash of very fine beaded fibres which pursue a tortuous course over the surface of the choroid plexus, being imbedded in a fine film of exudate which covers the choroid epithelium (Pl. 2, fig. 11). In the choroid plexus a relatively large, newly formed artery extends down towards the site of a localized lesion in the internal capsule which had evidently been produced at the time of operation. Alongside this artery, the regenerating nerve fibres have also extended their growth, and they finally beome lost in granulation tissue. So far as can be seen in the sections, the fibres which emerge from the implanted nerve are not accompanied by Schwann cells after leaving the granulation tissue immediately covering the cut end of the nerve. It may be noted that the epithelium of the implanted parotid duct has undergone considerable proliferation at one point, and has penetrated into the adjacent granulation tissue in a system of reticulating cords and scattered islands. At some places these epithelial outgrowths come into close contact with fibres of the white matter of the brain, and in two sections a few of these fibres are seen to be actually within an epithelial island.

*R* 228. Proximal stump of facial nerve inserted into brain and animal killed 1 week later. Pyridine-silver sections show the stump of the nerve passing through the parietal area of the cortex and the subcortical white matter, with its free end in contact with the fimbria of the underlying hippocampus. The fibres of the facial nerve have all undergone degeneration and no regenerating axons are present. The parotid duct had been inserted into the brain alongside the facial nerve and appears considerably dilated in the sections. The epithelial lining of the duct has undergone very active proliferation, penetrating in reticulating columns of cells into the adjacent cortex. This has led to degenerative changes in many of the fibres of the cortical plexuses which have been involved. At many points the invading epithelial strands, as well as the cellular reaction in the brain tissues, have displaced fibres of the cortical plexuses and have compressed them into compact fasciculi which might give the impression of an orientated growth of regenerating fibres. At other points in individual sections the network of invading epithelium seems to have cut off islands and recesses of cortical tissues in which many fibres of the cortical plexuses remain. Lastly, nerve fibres of the cortex are here and there actually found within the substance of epithelial clumps, lying among the constituent cells (Pl. 2, fig. 10). There is no evidence of the new growth of fibres of the brain in relation to the implanted portion of the facial nerve.

*R* 231. Proximal stump of facial nerve inserted into brain after crushing and animal killed 2 weeks later. The sections, treated by Bodian's protargol technique, show a condition very similar to that of the previous experiment. The nerve passes through the parietal cortex and penetrates into the underlying hippocampal formation. In one fasciculus only have any fibres of the facial nerve undergone regeneration, and here they are few and scattered. None of them can be traced as far as the cut end of the implanted nerve. There is no evidence of regenerative activity in intrinsic nerve fibres of the brain.

*R* 249. Distal stump of occipital nerve inserted into the brain and animal killed 3 weeks later. Pyridine-silver sections show that the nerve has traversed the occipital lobe where this overlaps the mid-brain and penetrates into the substance of the superior colliculus. There is very little reaction in the tissues of the brain except at the cut end of the implanted nerve where proliferated Schwann tissue has become extruded for a short distance beyond the limits of the epineural sheath into the damaged part of the colliculus. In this region there is some infiltration of immediately adjacent brain tissue with macrophages and an increased local vascularity. Damaged axons in the fibre plexus surrounding the stump of the implanted nerve are thickened, heavily impregnated and irregularly varicose (Pl. 2, fig. 12), and many of them end in large globular retraction bulbs (Pl. 2, fig. 13; Pl. 3, fig. 18). They show no evidence of active regeneration and no tendency to penetrate the implanted nerve or the proliferating Schwann tissue at its cut end. The implanted nerve is filled with healthy looking Büngner bands among which are scattered macrophages. Some fine actively growing fibres enter the implanted nerve

at its free extremity and can be traced along the elongated Schwann cells for a distance of just over 2 mm. (Pl. 2, fig. 14). Probably not more than four main axons have thus entered the nerve, but in their course they each divide into two or three separate fibres. Their precise origin cannot be determined. At the cut end of the implanted nerve they can be traced into the vascular tissue which is intermingled with proliferating masses of Schwann cells and here they become lost among intrinsic fibres of the superior colliculus which persist in the infiltrated zone. It may be noted that surrounding the lesion there is an appearance of fibres in the colliculus converging towards the stump of the implanted nerve (Pl. 2, fig. 12). This appearance is evidently the result of a secondary distortion produced by the lesion, and none of these fibres show any of the characteristic signs of active regeneration.

*R* 250. Distal stump of occipital nerve inserted into the brain and animal killed 4 weeks later. One week before death, the implanted nerve was cut close to the point where it enters the skull. Sections stained with the Bodian technique show that the nerve extends through the superficial cortex of the occipital lobe and the subcortical white matter, and penetrates into the cortex on the under surface of the occipital lobe (Pl. 3, fig. 15). The main cut end of the implanted nerve is actually imbedded in the deep cortical layers, and from it a compact outgrowth of Schwann tissue bulges out beyond the limits of the epineural sheath and presses against the upper surface of the superior colliculus. There is practically no inflammatory reaction in the surrounding brain substance, from which the nerve is separated only by a thin layer of delicate reticular tissue. The pial tissue overlying the cut end of the nerve is very vascular, and arterioles and capillaries can be traced from the under surface of the occipital lobe into the Schwann outgrowth. The implanted nerve appears in a healthy condition with a strong development of Büngner's bands. In a few sections newly growing axons are seen; they are extremely fine, usually beaded, and run a slightly tortuous course in close relation to elongated Schwann cells. In some cases they end in a conspicuous thickened bulb from which two or three fine sprouts extend out. A study of the serial sections show that probably not more than three separate main fibres have entered the implanted nerve, and they all lie in the same region near the surface. These fibres are found to penetrate the nerve from the proliferated Schwann tissue at the cut end. They can be traced along the implanted nerve for a distance of 1·2 mm. In one section a fine fibre can be seen curling round from the pial tissue on the under surface of the occipital lobe to enter the tip of the Schwann outgrowths, and it can be traced proximally to a plexus of apparently newly formed capillary vessels in the overlying pia. In another section a nerve fibre is seen entering the stump of the nerve alongside a small arteriole, dichotomizing as it does so in a manner characteristic of regenerating axons. The arteriole itself is derived from the vascular plexus in the pia, and it should be noted that in the tunica adventitia of some of the small arteries on the surface of the cortex vascular nerve fibres are distinctly impregnated. It thus appears almost certain that the few fine axons which have entered the implanted nerve are derived from vascular nerve fibres. There is no evidence that any of the intrinsic fibres of the white matter or cortex of the brain extend into or even towards the cut end of the implanted nerve. In the absence of inflammatory reaction, also, there is no distortion of the plexuses of the brain and there is also no tendency for orientation of their constituent fibres in the direction of the proliferated Schwann tissue.

*R* 254. Distal stump of occipital nerve inserted into the brain and animal killed 5 weeks later. Pyridine-silver sections show that the nerve passes through the occipital cortex and its cut end lies in the white matter of the subjacent subicular cortex. The tissues of the brain show a remarkable absence of inflammatory reaction and there is no apparent fibrosis around the nerve. The latter is filled with vigorous and healthy looking Schwann cells, neatly alined in characteristic band formation. From the cut end of the nerve, proliferated Schwann tissue fans out in a compact mass which presses against the adjacent cortex and reaches the pial tissue covering the under surface of the occipital lobe (Pl. 3, fig. 16). Under the high power, some of the Schwann cells appear to penetrate into the brain tissue to come into direct contact with fibres of the cortical plexuses. Others extend up along the superficial aspect of the epineural sheath between it and the surrounding brain tissue. Regenerating axons are scattered throughout the implanted nerve in somewhat surprising abundance. A study of the serial sections shows that they have grown downwards from the peripheral part of the nerve outside the skull, and they are therefore presumably recurrent fibres which may normally occur in the trunk of the occipital nerve. They can be followed down to the cut end of the nerve where they issue out into the proliferated mass of Schwann tissue. Here their further course is variable. Many become interwoven in a tangled skein within the 'Schwannoma' at the cut extremity of the nerve. Others, having emerged from the nerve trunk, turn back and run upwards on the surface of the epineurium for a considerable distance. One or two fine fibres penetrate into the pial tissues on the under surface of the occipital lobe, but their course here is very short. It may be noted that these regenerating fibres at many points come into the closest relation with intrinsic fibres of the adjacent cortex, but they never intermingle with them. The degree of impregnation of the material from this experiment is particularly favourable for following individual fibres section by section; yet in no instance could any fibre be found passing from the tissues of the brain into the implanted nerve. There is also no evidence of any orientation of fibres in the brain towards the Schwann proliferation. Further, it is to be remarked that the traumatic reactions of fibres in the brain which are so conspicuous in Exp. R 249 (such as thickening, heavy impregnation, varicosities and retraction bulbs) are almost completely absent in the present case. Presumably this is due to the longer interval which has allowed time for the resorption of fibres damaged at the time of the operation, and also to the almost complete absence of inflammatory reaction and cellular infiltration in the brain.

*R* 255. Distal stump of occipital nerve inserted into the brain and animal killed 6 weeks later. Pyridine-silver sections show that the nerve has traversed the occipital lobe of the cerebral hemisphere, and its free end lies in the pial tissue at the surface of the deep layer of cortex, in contact with the upper surface of the superior colliculus. The brain tissue in which the nerve lies shows almost no

reaction. The nerve is filled with closely packed Schwann cells in band formation, and but few macrophages remain (Pl. 3, figs. 17, 19). Extending along the nerve are many scattered fibres in process of regeneration, but they are considerably fewer than those in the previous experiment. By tracing these fibres through the serial sections they are all found to have travelled down in the nerve from outside the skull—that is, they are recurrent fibres. At the cut end of the nerve there is a small opening in the cap of fibrous tissue covering it, and though this is a slender outgrowth of Schwann cells, accompanied by a few regenerating fibres, has pushed its way towards the colliculus. The colliculus itself shows a circumscribed puncture lesion extending down for a few millimetres into its substance from the position of the free end of the nerve, which was evidently produced at the operation. Along the track of the lesion some fine strands of Schwann cells have penetrated into the colliculus and with them, also, a few of the regenerating fibres. The latter become extremely fine as they are traced down, until they pass beyond the limits of visibility. In immediate contact with this extension of Schwann tissue (with no intervening fibrous layer) are the fibre plexuses in the superficial layers of the superior colliculus. The component fibres appear inert (Pl. 3, fig. 20). Very occasionally a small retraction bulb is seen, and at the margin of the lesion a few fibres have become displaced by the proliferated Schwann cells, but there is no histological evidence of regenerative activity.

## DISCUSSION

The results of the experiments recorded above may be briefly discussed in relation to two main points —the behaviour of regenerating axons which grow down the inserted nerve towards the brain tissue, and the evidence for regenerative activity on the part of the intrinsic fibres of the brain.

The great majority of the regenerating fibres which grow down peripheral nerve stumps inserted into the brain do not extend beyond the confines of the implanted nerve and the compact mass of Schwann cells ('Schwannoma') which develops at its free extremity. Many are met by the obstruction of fibrous tissue at the cut end of the nerve and, running spirally around the inner surface of the perineural sheaths, form complex entanglements which often extend back up the nerve trunk for a little distance. Those fibres which enter the Schwann outgrowth at the cut end of the nerve in most cases become coiled round and interwoven with each other in a loose skein. Some regenerating fibres, however, may emerge from the cut end of the nerve and extend their growth into the surrounding tissues. Usually they turn aside from their original course, returning along the outer surface of the perineural sheaths of the implanted nerve and in immediate relation to the surrounding brain tissues. In this position regenerating fibres of the facial nerve have been observed running over the surface of the hippocampus and the optic tract, or alongside cut margins of cortical tissues or white matter of the brain. They may be separated from the latter by a thin secondary capsule, and in some cases they actually penetrate among the fibrous elements of this capsule. In one experiment there was a very conspicuous outgrowth of facial nerve fibres into a zone of granulation tissue in the adjacent part of the brain, and a significant feature in this case was the intermingling of the regenerating fibres with surviving fibres of the white matter. Another interesting observation is provided by the experiment in which regenerating facial nerve fibres had extended on to the choroid plexus in the lateral ventricle and had grown down in a convoluted course for some distance over the surface of the choroid epithelium. It may be noted that it was not possible to determine the presence of Schwann cells in relation to some of these finer extensions of the regenerating fibres. In no instance was any evidence found of regenerating facial nerve fibres penetrating into normal brain tissue in which no cellular infiltration was present.

Evidence which might indicate regeneration of intrinsic nerve fibres of the brain may be discussed under the following headings:

*Growth of fibres into the implanted nerves.* In two experiments only, in both of which the occipital nerve had been inserted into the brain, were newly regenerated axons found to enter the nerve from the brain tissues. In both these cases such regenerating fibres were very fine and extremely few—four main axons in one and three in another. In one case they were found to enter the Schwann outgrowth in company with small blood vessels from the pial tissue on the surface of the brain, and it was apparent that they were extensions of the vascular nerve fibres found in the tunica adventitia of cerebral arteries. In the other case they were traced proximally to newly vascularized tissue at the site of a lesion in the superior colliculus, and, though their precise origin could not be demonstrated, the sections provided no evidence that they had been derived from the intrinsic fibres of the brain. On the contrary, it seems probable that in this case, also, they had regenerated from vascular fibres. It is concluded, therefore, that the growth of new axons from the brain into the implanted nerves which was observed in these experiments almost certainly involves only extrinsic fibres accompanying blood vessels. This accords with observations previously made on predegenerated nerve grafts (Clark, 1942).

*Orientation of intrinsic fibres of the brain towards the implanted nerves.* In the previous communication, attention was drawn to Tello's observation (1911) that fibres of the white matter of the host brain converged towards an implanted fragment of predegenerated nerve as though attracted towards it, and to his inference that this appearance indicated a directed growth under the influence of the grafted tissue. The observations of Tello were

confirmed, but it was suggested that the orientation of the fibres is partly the result of a traction effect imposed on the host tissue by the graft, and partly due to the dislocation of pre-existing fibres by the infiltration of the brain with granulation tissue. In the present series of experiments, a similar orientation of fibres has been found only in those cases in which local inflammatory reaction of the brain is marked, and a study of the sections makes it clear that the fibres are not in a state of active regeneration but are pre-existing fibres distorted by the initial lesion or by cellular infiltration. In some cases remains of the fibre plexuses of the brain had been compressed into compact and apparently orientated fasciculi between small masses of granulation tissue, or, in those experiments in which the parotid duct had been inserted with the nerve, by proliferating masses of epithelial cells. In other cases the survival of fibres of the brain plexuses alongside blood vessels in the infiltrated tissue also gives rise to an appearance of orientated growth which is certainly fallacious. Lastly, it must be emphasized that, in those experiments in which there is little or no damage to or reaction in the surrounding brain tissue, no orientation of the intrinsic fibres of the brain towards the implanted nerve or the Schwann proliferation is found. Since in these cases the Schwann elements of the implanted nerve are seen histologically to be in a vigorous and active condition, it may be assumed that they are not capable by themselves of exerting an attractive influence on the intrinsic fibres of the brain.

*Morphological characters of fibres of the brain immediately adjacent to the implanted nerves.* As in the previous series of experiments, fibres of the brain in immediate relation to the implanted nerve have in some cases been found to show striking traumatic changes in their detailed configuration, such as thickening, irregular varicosities, end-swellings, deviations from their normal direction, granulation and so forth. There is little doubt that some of these changes have occasionally been regarded by neurohistologists as evidence of regeneration, or at least of attempted or obstructed regeneration. However, there is no clear reason to suppose this is so. Retraction bulbs are well recognized to be degenerative phenomena (Cajal, 1928), and they are usually to be distinguished from thickenings resulting from obstruction at the advancing ends of regenerating fibres by their coarseness and their globular outline. The varicosities of degenerating axons often resemble the beading of growing axons, but they are commonly less regular and less sharply outlined. Lastly, the displacement of nerve fibres by the immediate pressure of cellular proliferation and infiltration is to be distinguished from the aberrant wanderings of newly regenerated fibres. It should be noted that, in the experiments in which the implanted nerve had been left in position for 5 and 6 weeks, and in which the surrounding brain tissue had suffered minimal damage and showed an almost complete absence of inflammatory reaction, the fibres of the brain adjacent to the nerve were almost completely devoid of irregularities which might be taken as evidence of regenerative activity.

*Relation of fibres of the brain to epithelial proliferation.* It has been noted that in those experiments in which the parotid duct had been inserted into the brain alongside the facial nerve, the epithelial lining of the duct shows considerable proliferative activity, sending out reticulating columns of cells which invade the adjacent cortical and white matter of the brain. Further, nerve fibres derived from those of the brain are in a few sections found to be situated within epithelial clumps, among their constituent cells. This appearance might be taken as evidence of regenerative growth on the part of the brain fibres, leading to their active penetration into the epithelial masses. However, a careful study of the material makes it reasonably certain that they are really pre-existing fibres which have become secondarily caught up and included within the advancing columns of cells. Where the latter are found to contain nerve fibres, the cellular elements are somewhat loosely arranged, and the histological appearance makes it easy to understand how the rapidly proliferating epithelium is able to surround and occasionally enclose a few fibres. The latter, it should be noted, show none of the characteristic morphological features of regenerating fibres. On the contrary, they are clearly in process of degeneration for many of them are fragmenting and are accompanied by a granular argentophil deposit, while others terminate in swollen retraction bulbs. In connexion with these observations, reference may be made to experiments by Shirai (1935) in which pieces of skin were transplanted into the brains of rabbits. This author believed that active regeneration of nerve fibres in the brain occurred, leading to penetration and innervation of the transplanted skin, sometimes with the formation of a differentiated end-apparatus. However, from his descriptions and photomicrographs, it is hardly possible to avoid the conclusion that the fibres observed by him were similar to those seen in relation to the proliferating epithelium in the present series of experiments. Secondary inclusion was evidently mistaken for active penetration, and retraction bulbs were mistaken for differentiated end-formations. The fact that Shirai found that all the 'regenerating' fibres had disappeared after 100 days further supports this interpretation.

The results of all the experiments recorded in this report reinforce the general impression gained from the previous series of experiments that intrinsic fibres of the rabbit's brain probably do not possess the capacity for true regeneration. Clearly it is a matter of great difficulty completely to prove that this is the case, since it remains possible that under experimental conditions other than those which

have hitherto been employed regenerative growth might possibly occur. There is reason to believe, however, that in the present series of experiments optimal conditions for inducing regeneration of nerve fibres in the brain have been provided; the implanted nerves were in effect autografts in which a vascular supply was maintained, the Schwann elements showed normal and healthy activity and came into the closest relation with the nerve fibres of the brain, in most cases the surrounding brain tissue showed remarkably little inflammatory reaction, and the inserted nerves clearly provided perfectly good facilities for a vigorous regeneration of extrinsic fibres growing downwards towards the brain. In view of these considerations it may be accepted that the negative evidence presented by the experimental material is highly significant.

In general, in the assumption that the fundamental identity in structure of axons in the central and peripheral nervous system implies a similar capacity for regeneration under suitable conditions, two explanations are commonly put forward to account for the normal absence of regeneration in the brain and spinal cord of higher vertebrates during post-natal life. One is the absence in the central nervous system of Schwann cells which are believed to facilitate regeneration either by some chemotropic action or by the provision of suitable surfaces along which the growth of axons can proceed. The other is the density and texture of the tissues of the central nervous system, which impose an insuperable obstacle to fibres which may be attempting regeneration. In regard to the presumed influence of the Schwann cells, it may be emphasized again that at many places in the experimental material these elements were seen to be in direct contact with fibre plexuses of the brain, yet these fibres showed no tendency to extend towards or along them. The same lack of response was shown in places where Schwann bands, accompanied by actively growing peripheral nerve fibres, were found to pass in the closest relation to the cortex and white matter of the brain with no intervening barrier of fibrous tissue that could be defined histologically. The probability that the tissues of the central nervous system are not suitable for the growth through them of regenerating nerve fibres in post-natal life is indicated by the fact that in no case were the regenerating fibres growing down the implanted nerves found to penetrate into normal brain tissue. On the other hand, facial nerve fibres were found to extend out into zones of cellular infiltration still containing persistent fibres of the white matter (freely intermingling with the latter), or on the surface of the choroid plexus in the lateral ventricle, or on the surface of the optic tract and hippocampus, and also among the fibrous elements of secondary capsules which had been formed in the brain tissues immediately surrounding the implanted nerve. Similar paths were of course available for the outgrowth of injured fibres of the brain if the latter are indeed capable of regeneration. It may be concluded, therefore, that the normal absence of regeneration in the central nervous system is fundamentally due neither to the absence of Schwann cells nor to the physical obstruction provided by the texture of the nervous matrix. In regard to the second factor, attention may be drawn to a study made by Clark (1914) on nerve regeneration in fowls following experimental non-traumatic degeneration. The degeneration was produced by prolonged feeding on polished rice, and regeneration by a return to an adéquate diet. While new outgrowths of axons took place in the peripheral nerves, no regeneration occurred in the spinal cord. Yet the glial sheaths of individual nerve fibres in the cord, containing globules of degenerated myelin, apparently persisted for more than a year, and it seems that these sheaths would provide adequate pathways for new outgrowths of axons if the intrinsic fibres of the spinal cord were capable of regeneration.

## SUMMARY

In a series of rabbits the proximal stump of the cut facial nerve or the distal stump of a cut occipital nerve was inserted into the brain and left in position for 1 to 6 weeks. As compared with the implantation of homografts of predegenerated sciatic nerves recorded in previous experiments, this method combines the advantages of an autograft with the retention of at least a part of the vascular supply. Reaction in the tissues of the brain was reduced to a minimum, and it is claimed that the optimal conditions were provided for the induction of the growth of intrinsic fibres of the brain if these are capable of regeneration like the peripheral nerve fibres. In some experiments proliferated Schwann tissue had extended out from the cut end of the nerve into the adjacent tissues of the brain. These Schwann outgrowths may be accompanied by regenerating fibres which had grown down in the implanted nerve from outside the skull. Such regenerating peripheral nerve fibres may intermingle freely with intrinsic fibres of the brain, in areas of cellular infiltration. They have also been observed extending up the outer surface of the perineural sheath of the nerve, over the surface of the optic tract and the hippocampus, and also over the surface of the choroid plexus in the lateral ventricle. In two experiments three or four regenerating axons of very fine diameter were found to have entered the cut end of the implanted nerve from the brain tissues, but these were almost certainly derived from vascular nerves accompanying cerebral blood vessels, and not from intrinsic fibres of the brain. In none of these experiments was any evidence found of regenerative activity on the part of the nerve fibres of the brain, in spite of the fact that they may come into the closest relation with

actively proliferating strands of Schwann cells and with regenerating peripheral nerve fibres. As in previous experiments, some sections gave the appearance of a convergence towards the end of the implanted nerve of fibres in adjacent regions of the brain. This appearance, however, was not found in the absence of traumatic reaction in the tissues of the brain, and it is believed to be the result of dislocation of pre-existing fibres and not indicative of active growth. In some experiments in which the parotid duct had been inserted into the brain along with the facial nerve, the proliferating epithelium of the duct had invaded the adjacent tissues of the brain. No evidence was found of the regeneration of fibres of the brain in relation to the epithelial masses. The negative evidence of the histological material is believed to justify the conclusion that the intrinsic fibres of the rabbit's brain show no capacity for regeneration under the conditions of the experiments.

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## EXPLANATION OF PLATES

### Plate 1

Fig. 1. The stump of the facial nerve, inserted into the brain 3 weeks previously (R 230, 28·6). The cut end of the perineural sheath is marked *a*, and from it numerous fasciculi of regenerating axons are seen streaming out into an area of granulation tissue which infiltrates the adjacent region of the brain. Above (*b*) are seen the fibres of the white matter of the brain; they show no regenerative activity in relation to the outgrowth of facial nerve fibres. × 130.

Fig. 2. A higher power view of the regenerating fibres seen in the previous figure (R 230, 26·10). The fibres are seen to be extending along strands of Schwann cells which have grown out from the cut end of the nerve into the granulation tissue. × 380.

Fig. 3. Some of the terminal sprouts (*a*) of the regenerating facial nerve fibres in the same experiment (R 230, 26·9). Above and to the right are seen numerous persisting fibres of the white matter of the brain; these show fusiform varicosities but no signs of regenerative activity. The intimate relation between the brain fibres and the actively growing fibres of the facial nerve should be noted. × 380.

Fig. 4. A fasciculus of regenerating fibres (*a*) in R 230 (27·3), extending along a strand of Schwann cells in close relation to fibres of the white matter of the brain (*b*). The latter show no tendency to grow out in relation to the regenerating fibres. × 430.

Fig. 5. Longitudinal section of one of the fasciculi of the facial nerve in R 230 (27·6), showing the usual fate of the regenerating fibres. At the end of the perineural sheath they form a tangled skein from which many fibres extend backwards again, winding in a spiral manner on the inner aspect of the sheath. × 200.

Fig. 6. This illustrates a skein of fine, actively regenerating fibres of the facial nerve (*a*) growing along the surface of the hippocampus in the region indicated by the right arrow in Pl. 2, fig. 1 (R 233, 34·10). To the left are seen the alvear fibres at the surface of the hippocampus. × 360.

### Plate 2

Fig. 7. The stump of the facial nerve in R 233 (40·4), projecting down on the medial aspect of the cerebral hemisphere between the optic tract (*a*) and the hippocampus (*b*). Fasciculi of regenerating fibres can be seen in the stump. The arrows mark the regions where fibres after growing out from the cut end of the nerve have extended up on the surface of the hippocampus (see Pl. 1, fig. 6) and on the surface of the optic tract (see Pl. 2, fig. 9). × 32.

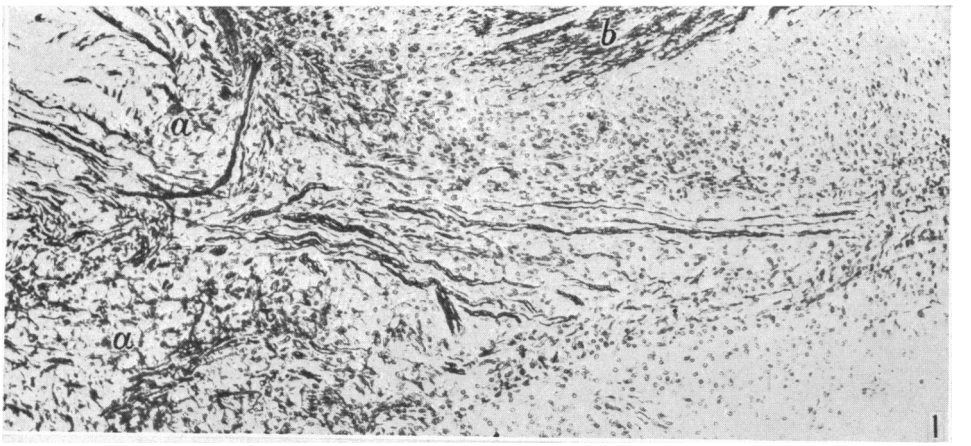
Fig. 8. R 233 (39·10). A small fasciculus of regenerating facial nerve fibres (right) passing in close proximity to the fibres of the white matter of the brain (left). The latter show no tendency to extend out towards the regenerating fibres. × 230.

Fig. 9. High power view from R 233 (41·10) showing regenerating facial nerve fibres (centre and right) growing along the surface of the optic tract (*a*), in the region indicated by the left arrow in Pl. 2, fig. 7. × 240.

Fig. 10. A proliferating mass of parotid duct epithelium in R 228 (20·8). In the centre a nerve fibre is seen extending up into the mass, and to the right is a small fasciculus of fibres in close relation to the epithelial cells. These fibres show no histological characteristics of active regeneration; they are evidently pre-existing fibres which have become secondarily included in the proliferating columns of cells. × 360.

Fig. 11. A skein of fine regenerating fibres of the facial nerve in R 229 (28·8), which have grown down on the surface of the choroid plexus in the lateral ventricle. They are imbedded in a thin film of exudate which covers the choroid epithelium. × 230.

Fig. 12. Section from R 249 (25·9), showing the relation of the proliferated Schwann tissue at the extremity of the implanted occipital nerve to the fibres of the superior colliculus. Below and to the right are seen the bands of Schwann cells. At the margin of the Schwann tissue the fibres of the superior colliculus stop abruptly and show no tendency to invade the tissue. Many of them show traumatic reactions such as irregular thickenings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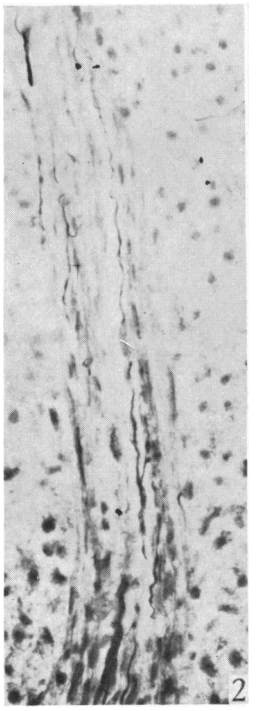
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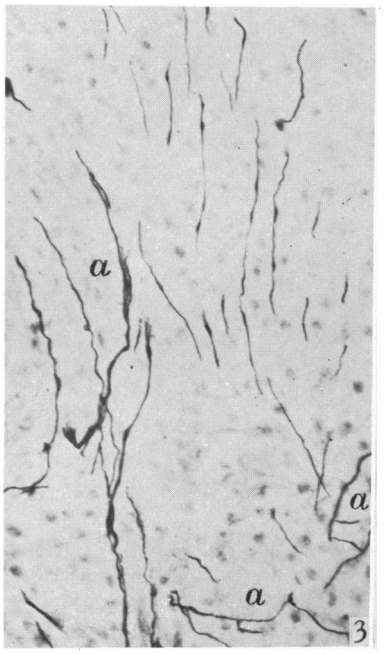
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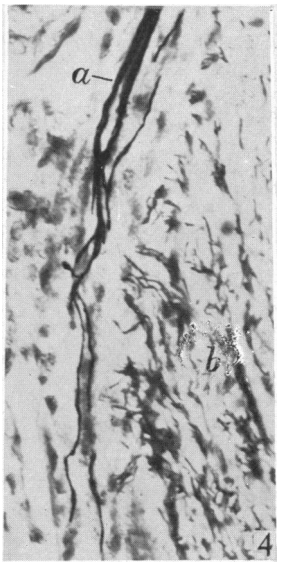
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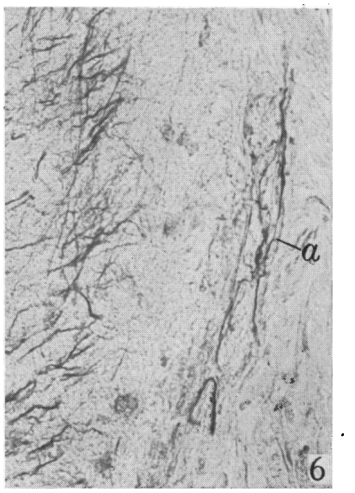
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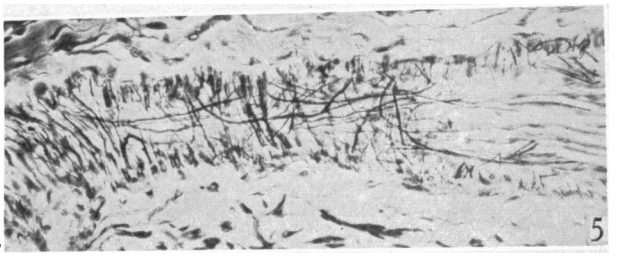
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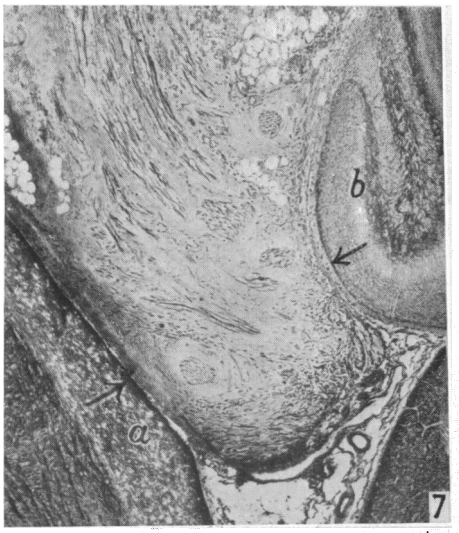
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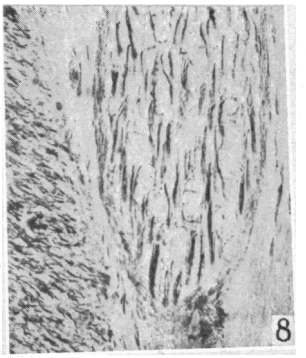
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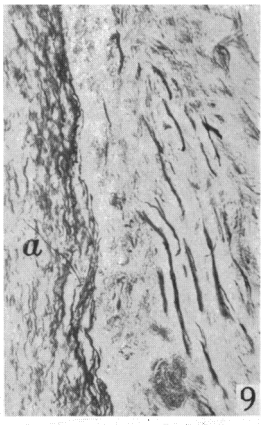
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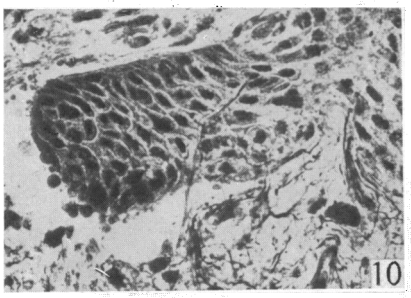
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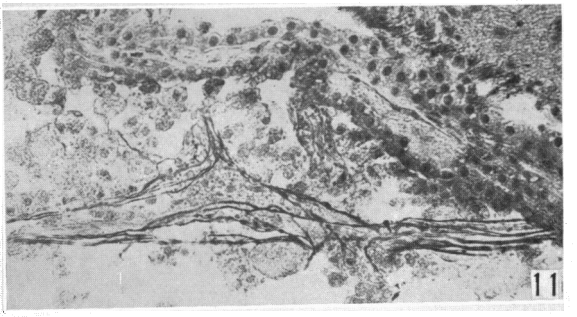
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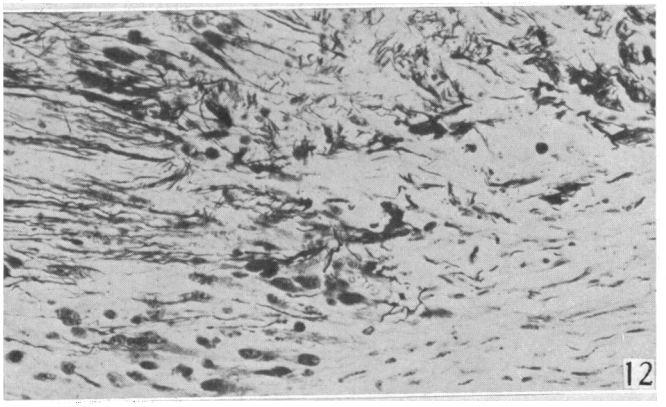
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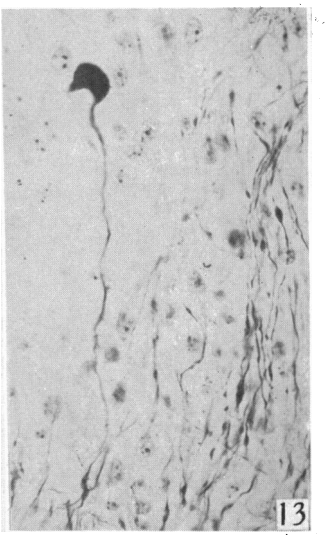
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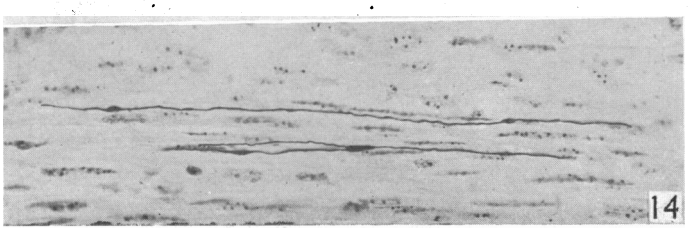
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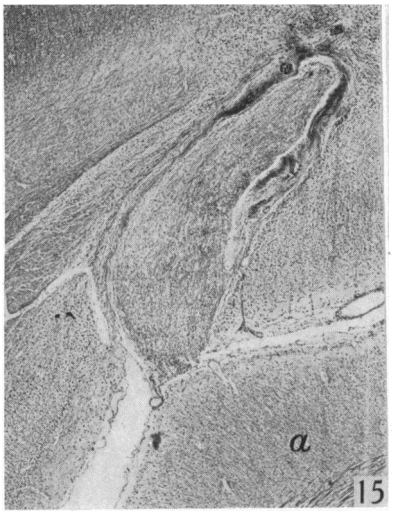
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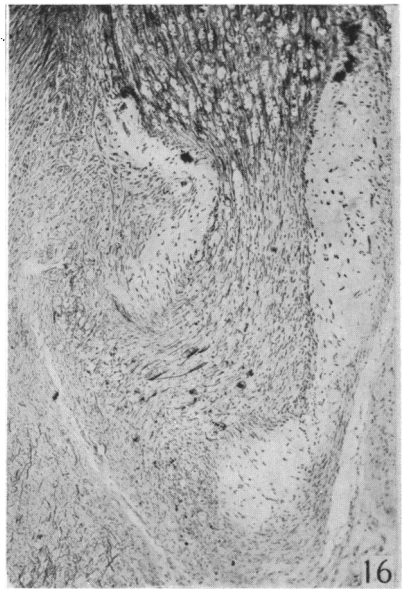
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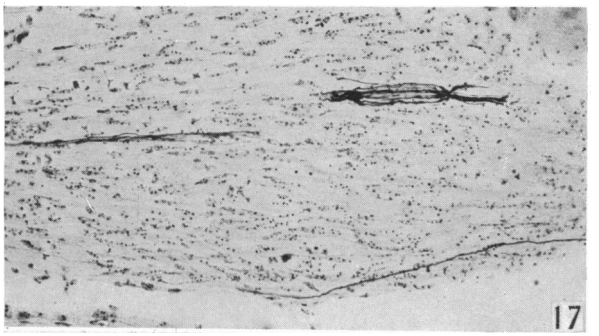
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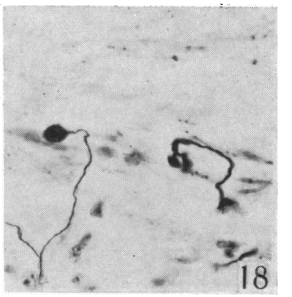
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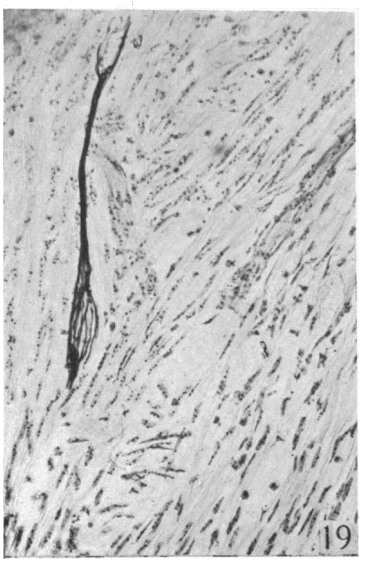
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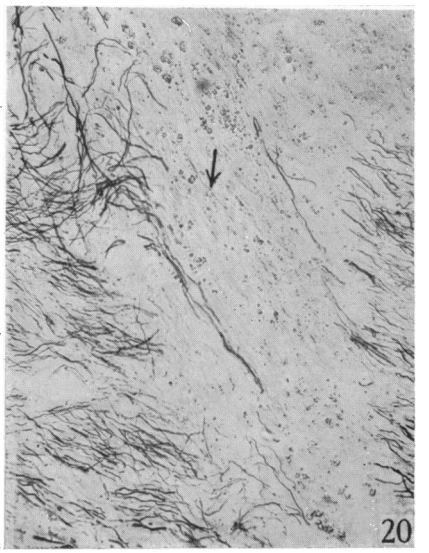
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and retraction bulbs. To the left the fibres of the superior colliculus seem to stream towards the outgrowth of Schwann cells. This is a secondary distortion produced by the lesion, and none of the fibres show histological characters of active regeneration. × 290.

Fig. 13. High-power view showing traumatic reaction in fibres of the superior colliculus in R 249 (22·2). A fibre is seen ending in a conspicuous retraction bulb at the margin of the Schwann outgrowth (above). To the right many traumatized fibres are shown with irregular varicosities. × 410.

Fig. 14. Two fine regenerating fibres in the implanted occipital nerve in R 249 (21·10). They have grown into the nerve from the brain tissues and are almost certainly derived from vascular nerves. The fibres can be seen extending along in close relation to Schwann cells. They show beading, and the lower fibre dichotomizes at a local thickening. × 410.

PLATE 3

Fig. 15. Low-power view of the stump of the occipital nerve in R 250 (14·5), imbedded in the cortex. The Schwann outgrowth at its free end comes into contact with the upper surface of the superior colliculus (*a*). × 24.

Fig. 16. The free end of the occipital nerve in R 254 (13·5). Above is seen the stump of the nerve, and from it a mass of proliferating Schwann tissue has grown out into contact with the adjacent brain tissue. In the Schwann outgrowth can be seen some regenerating nerve fibres which have grown down in the nerve from outside the skull. Below and to the left some fibres of the cortical plexuses are visible. They show no tendency to invade the Schwann tissue. × 58.

Fig. 17. Section of the implanted occipital nerve in R 255 (23·8). Note the closely packed Schwann cells, and some regenerating fibres which have grown down into the nerve from outside the skull. × 240.

Fig. 18. Fibres showing traumatic reactions at the margin of the Schwann outgrowth in the superior colliculus in R 249 (22·9). The fibre to the left shows a globular retraction bulb, and that to the right is markedly thickened. The nuclei of Schwann cells can be faintly discerned above. × 410.

Fig. 19. Schwann bands in the occipital nerve in R 255 (22·8), 6 weeks after its implantation. Macrophages have now mostly disappeared. A fasciculus of regenerating fibres is seen; these have grown down in the nerve from outside the skull. × 240.

Fig. 20. Section through a puncture lesion in the superior colliculus in R 255 (22·14). The site of the lesion is occupied by granulation tissue into which a few Schwann cells from the cut end of the implanted occipital nerve have penetrated. Accompanying these cells are some extremely fine regenerating fibres which have grown down in the nerve from outside the skull. The position of one of these fibres is indicated by the arrow. On either side of the lesion are seen fibres of the superior colliculus. Some have been displaced by the lesion, but none of them show any signs of regeneration. Note that now (6 weeks after implantation) traumatic reactions of individual fibres have disappeared. × 160.